Comparison of the T Cell Patterns in Leprous and Cutaneous Sarcoid Granulomas

Presence of V α 24-Invariant Natural Killer T Cells in T-Cell-Reactive Leprosy Together with a Highly Biased T Cell Receptor V α Repertoire

Martin Mempel,^{*,†} Beatrice Flageul,[†]
Felipe Suarez,^{*} Catherine Ronet,^{*}
Louis Dubertret,[†] Philippe Kourilsky,^{*}
Gabriel Gachelin,^{*} and Philippe Musette^{*,†}

From the Institut Pasteur,^{*} Unité de Biologie Moléculaire du
Gène, INSERM U277, Département d'Immunologie, Paris; and the
Institut de Recherche sur la Peau,[†] INSERM U312, l'Hôpital
St.-Louis, Paris, France

The T-cell-reactive (eg, tuberculoid and reversal) forms of leprosy represent a well-defined granulomatous reaction pattern against an invading pathogen. The immune response in cutaneous sarcoidosis is a granulomatous condition that pathologically is very similar to T-cell reactive leprosy. However, it lacks a defined causative agent. In view of the role of NKT cells in murine granulomas induced by mycobacterial cell walls, we have searched for the presence of NKT cells in the cutaneous lesions of both leprosy and sarcoidosis. These cells were present in T-cell-reactive leprosy but were undetectable in cutaneous sarcoidosis. We have also studied the TCR V α repertoire in the two diseases. In addition to V α 24⁺ NKT cells, all patients with T-cell-reactive leprosy showed a very restricted T-cell-reactive V α repertoire with a strong bias toward the use of the V α 6 and V α 14 segments. V α 6 and V α 14⁺ T cells were polyclonal in terms of CDR3 length and J α usage. In contrast, most sarcoidosis patients showed a diverse usage of V α chains associated with clonal or oligoclonal expansions reminiscent of antigen-driven activation of conventional T cells. Thus the origin and perpetuation of the two kinds of granulomatous lesions appear to depend on altogether distinct T-cell recruiting mechanisms. (*Am J Pathol* 2000, 157:509–523)

The formation of granulomas represents a particular defense mechanism aimed at preventing the spread of a potentially hazardous organism that has invaded a tissue

compartment. Granulomas are the histological hallmark of infections with mycobacteria and are well known as a clinical manifestation of leprosy. In this disease, the immune response varies from the unreactive, T-cell-poor, multibacillary form of polar lepromatous leprosy (LL) with loosely organized granulomas to the T-cell-rich, paucibacillary forms of T-cell reactive leprosy (eg, polar tuberculoid (TT)) with well-organized granulomatous structures. Reversal leprosy (RR) represents an acute cellular immune response against *Mycobacterium leprae*, which may correspond to the early events of T cell reactivity.

Pathologically, granulomatous reactions highly similar to the T-cell-reactive forms of leprosy are found in diseases of unknown origin, such as sarcoidosis. Many cell types of the innate and adaptive immune systems participate in the elaboration of cutaneous granuloma formation in both diseases, with the typical feature of a central accumulation of histiocytes surrounded and infiltrated by lymphocytes. The lymphocytic rim is composed of $\alpha\beta$, $\gamma\delta$ T lymphocytes and occasionally NK cells. These cells invade the site of granulomatous inflammation after a variety of stimuli, including antigenic ones, such as mycobacterial peptides, lipoproteins, and glycolipids,^{1–8} or chemoattraction by cytokines and chemokines.^{9–11} In view of the pathological similarity of the two kinds of diseases, a common etiology has been suggested, inasmuch as sarcoidosis represents the inflammatory response to an as yet unidentified microorganism, most probably of mycobacterial origin.^{12–14} The causative association of mycobacteria and sarcoidosis is still a focus of controversial discussions.^{15,16}

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Address reprint requests to Dr. G. Gachelin, Département d'Immunologie, Institut Pasteur, 25 rue du Dr. Roux, 75015 Paris, France. E-mail: ggachel@pasteur.fr.

Over recent years the T cell response to mycobacteria has been extensively studied and found to display original features; the response to various antigens, and particularly to mycobacterial glycolipids, is well documented.^{17,18} In an animal model, NKT cells, a CD1d-restricted T-cell subpopulation with very unique features,¹⁹ have been found to be recruited by mycobacterial glycolipids.²⁰ Moreover, in this model, NKT cells are needed for the granulomatous lesions to develop. These NKT cells, however, have to our knowledge not yet been identified in human mycobacteria-induced granulomas.

One way to test the "mycobacterial" origin of sarcoidosis is to analyze and compare the T-cell populations and the T-cell receptor usage in cutaneous mycobacteria-induced granulomas such as leprosy, and in the cutaneous granulomatous lesions of sarcoidosis, as performed for pulmonary patients in earlier studies.²¹⁻²³ As cutaneous manifestations are currently found in a subgroup of sarcoidosis patients, the comparison of cutaneous sarcoidosis and leprosy lesions offers a convenient model for the study of the characteristics of granulomas in identical tissue compartments and without the difficulties of pulmonary specimen sampling or the bias of using bronchoalveolar lavage fluids.

Using a combination of immunohistochemistry, polymerase chain reaction (PCR)-based technology, and DNA sequencing, we have analyzed the T-cell population in human leprosy patients and compared them to cutaneous sarcoidosis. The present data lead to the conclusion that the two kinds of granulomas, although histologically similar, are composed of different T-cell populations and argue against a mycobacterial origin of sarcoidosis.

Materials and Methods

Patients Included in the Study

The diagnosis of leprosy and sarcoidosis was confirmed by clinical and histological criteria. Leprosy patients were classified according to the criteria of Ridley and Jopling into polar tuberculoid (TT), borderline tuberculoid (BT), polar lepromatous (LL), borderline lepromatous (BL), and midborderline (BB).²⁴ A total of five patients with tuberculoid leprosy (TT, BT), three patients with lepromatous leprosy (LL, BL), two patients with the reversal form of leprosy (RR), and six patients with cutaneous sarcoidosis were included in the study. Furthermore, the skin biopsy of one healthy individual undergoing plastic surgery served as the control for unaffected skin. After informed consent was obtained, 4-mm lesional punch biopsies were taken in local anesthesia and snap-frozen in liquid nitrogen, and specimens were cut into two fragments for immunohistochemistry and molecular biology analysis, respectively. A summary of the included patients is shown in Table 1.

Immunohistochemical Staining

Frozen sections were analyzed for the expression of CD3, CD4, CD8, CD16, CD56, CD57, CD68, panTCR $\alpha\beta$, and

Table 1. Summary of Patients

Patients	Sex/ age (yr)	Affected organ	Treatment at the time of biopsy
T1	M/13	Skin	None
T2	F/67	Skin	None
T3	M/14	Skin	None
T4	F/53	Skin	None
T5	M/22	Skin	None
R1	M/75	Skin	PCT*
R2	F/41	Skin	PCT*
L1	M/42	Skin	None
L2	M/56	Skin	None
L3	M/25	Skin	None
S1	F/40	Skin	None
S2	M/47	Skin	None
S3	M/40	Skin	None
S4	F/40	Skin, lungs, liver	None
S5	F/31	Skin	None
S6	F/60	Skin	None
Ctrl	F/40	Skin	—

*PCT, Antibacillary polychemotherapy (rifampin, dapsone, clofazemine).

panTCR $\gamma\delta$ (Dako, Trappes, France); CD1a, CD1b, CD1c, CD83, and V α 24 (Immunotech, Marseille, France); and CD1d (Biosource, Camarillo, Calif., USA), using commercially available monoclonal antibodies, and a streptavidin-biotin immunoperoxidase technique, using 3-aminoethylcarbazole to produce positive brownish staining (Beckman-Coulter, Paris, France). For staining with anti-V α 24 we used a noncoupled primary antibody, followed by a biotin-labeled secondary antibody to reduce non-specific background staining.

The percentage of positive cells was calculated by determining the number of stained cells among the total number of cells within the granulomas (eg, hematoxylin-stained nuclei).

As control samples, healthy skin from patients undergoing plastic breast surgery were used to test the staining pattern in unaffected skin.

RNA Extraction and cDNA Preparation

Specimens were disrupted in Trizol (Gibco) with a Polytron homogenizer. RNA extraction and cDNA preparation were carried out according to standard protocols, using AMV reverse transcriptase (RT) from Boehringer Mannheim (Mannheim, Germany).

TCR α -Chain Analysis

The "Immunoscope" technique for determining complementary region 3 (CDR3) length and distribution has been described elsewhere.²⁵⁻²⁷ Briefly, standardized amounts of cDNA (ie, the product of the reverse transcription of 10 μ g of total RNA) were PCR amplified, using each of the 29 V α -specific probes and a common C α -specific probe. Each V α -C α PCR product was analyzed by electrophoresis in an agarose gel. For CDR3 length diversity, PCR-amplified products were submitted to five cycles of primer extension, using an internal, fluorescent, C α -specific probe. The labeled material was

loaded on a sequencing gel and analyzed with an automatic sequencer (Applied Biosystem) equipped with a computer program (Immunoscope; Applied Biosystems) that enables the determination of the intensity of fluorescence of each band as well as its actual size. The results are depicted as peaks, the surfaces of which are proportional to the amount of material and the locations of which are dictated by the length of the CDR3 region. The size distribution of the $V\alpha$ - $C\alpha$ is Gaussian in the case of non-activated or polyclonally activated lymphocytes, whereas proliferating T cells generated a non-Gaussian distribution with amplified peaks corresponding to clones with a definite CDR3 length within a $V\alpha$ - $C\alpha$ combination.

Primer and PCR Conditions and Detection of Invariant $V\alpha$ Chains

To amplify the $V\alpha$ repertoire of the invaded T cells we used a panel of 29 $V\alpha$ -specific primer together with a $C\alpha$ -specific primer as well as an internal $C\alpha$ run-off primer, which have previously been described.²⁶ The run-off primer for the J α 33 (5'-CCAGATTAAGTATAGTTGCTATC-3'), the J α 29 (5'-AAGAGGTGTGTTTCTACGTC-3'), the J α 48 (5'-TAATTTCTCATTTCCAAAGTT-3'), and the J α 18 (5'-GCCTCCCCAGGGTTGAGCCTCTG-3') clonotypes were designed according to the publications of Porcelli et al and Han et al.^{28,29} Primers for human CD3 and CD1d were as follows: CCAGGCTGATAGTTCGGTGA (CD3-5') and TGTCTGAGAGCAGTGTCCAC (CD3-3') and AGCCTGTATGGGTGAAGTGG (CD1d-5') and TAAAGCCCACAATGAGGAGG (CD1d-3'). For the primary PCR reaction cDNA was amplified in 40 cycles (30' 94°—30' 60°—30' 72°) followed by five cycles (30' 94°—30' 60°—30' 72°) with the fluorescent run-off primer. To exclude a higher affinity of individual primers as a reason for a positive amplification product, various primers were tested in a kinetics of 20/25/30/35 and 40 cycles with a positive control of peripheral blood mononuclear cell (PBMC) cDNA of a healthy individual in which all $V\alpha$ chains had been found to be amplified.

Direct DNA Sequencing

Direct sequencing of PCR products was carried out with a Sequenase kit (USB-Amersham, Cleveland, OH) according to the manufacturer's instructions, after separation of the PCR products by electrophoresis in a 2% agarose gel and electroelution of the proper DNA fragments.

Cloning and Sequencing of PCR Products

PCR products were ligated into a commercially available vector (Zero blunt; Invitrogen, Groningen, the Netherlands) and transformed into *Escherichia coli* (Invitrogen). Sequence analysis was carried out according to standard protocols (Perkin Elmer) and analyzed with ABI-Prism 373 software (Applied Biosystems).

Results

Main Histological Features of the Leprosy and Sarcoidosis Biopsies Used in the Present Study

Biopsies were frozen, sectioned, and immunostained. We first determined the number of macrophages, dendritic cells, and NK lymphocytes, using simple staining for CD68, CD1a, CD16, CD56, CD57, and CD83 molecules, respectively. The staining pattern showed comparable numbers of cells within the T-cell-reactive forms of leprosy and sarcoidosis patients, whereas lepromatous leprosy patients showed no or only weak staining for the analyzed cell types, with the exception of CD68, which stained positive in all lepromatous leprosy patients, confirming the presence of macrophages within the lesions (data not shown). In addition, comparable numbers of $\gamma\delta$ T cells as well as a similar ratio of CD4⁺ to CD8⁺ cells were detected in TCR leprosy as well as in sarcoidosis. Lepromatous leprosy patients showed only poor infiltration by any of the investigated T-cell subpopulations. The identified T cells dominated in both types of granuloma (sarcoidosis and leprosy) in the areas surrounding the central epithelioid cell accumulation. The immunohistochemical features of the samples under study are in agreement with previous findings showing that the composition of granuloma infiltrating immunocytes in cutaneous sarcoidosis is similar to that of T-cell-reactive leprosy.⁴

In view of the involvement of CD1 molecules in the response to mycobacterial antigens, the expression of CD1a–d molecules was examined. Few or no CD1a⁺, CD1b⁺, CD1c⁺, or CD1d⁺ cells were found in lepromatous leprosy patients, but remarkable levels of expression of CD1a, b, c were found in all TCR leprosy patients, as reported in a previous publication.³⁰ To our surprise, even higher numbers of CD1b⁺ cells and a remarkable expression of the other CD1 molecules was found within the investigated sarcoidosis patients (Figures 1 and 2). The positive staining cells in both diseases accumulated within the dermal granulomas, with the exception of CD1a, which, in addition, constantly stained positive for epidermal cells, most probably because of the presence of epidermal dendritic cells. This epidermal staining pattern was also seen in healthy control biopsies. CD1b and CD1c stained positive for epidermal cells only occasionally, without showing associations with one of the investigated disorders. Some of the biopsies showed positive staining of CD1d, also in the basal layer of the epidermis, suggesting an expression of this molecule by human keratinocytes, as described earlier,³¹ but this pattern was seen for leprosy and sarcoidosis patients and was also found in the uninvolved skin of healthy control subjects. The positive staining pattern was paralleled by the detection of CD1d-RNA by RT-PCR in all sarcoidosis and TCR leprosy patients (data not shown).

To define the nature of the CD1d⁺ cells, for some of the patients we used serial sections that were stained with various cell surface markers. Using this technique CD1d⁺ cells within the granulomas colocalized with CD83⁺ cells, suggesting dendritic cells as the predominant CD1d⁺ cell population within the granulomas. In

Expression of CD1 molecules in leprosy and sarcoidosis patients

Figure 2. Quantification of CD1⁺ cells in leprosy and sarcoidosis. Positive cells in the granulomas were counted and are expressed as a percentage of the total cell numbers within the analyzed field (eg, 100 cells). Whereas lepromatous leprosy patients (L) showed no or only exceptional positive staining (CD1b) with the investigated molecules, sarcoidosis (S) and T-cell-reactive leprosy patients (T, R) yielded expression of all CD1 molecules in part even at higher levels for sarcoidosis biopsies (CD1b, CD1c). *Staining for CD1d could not be performed in all patients because of limitations in biopsy material, but RT-PCR for CD1 showed positive results in all T/R and S patients.

addition, staining for CD1d was strong in the central areas of the granulomas and only weak for the periphery of the granulomas where CD3 T lymphocytes dominated, thus rendering unlikely a major contribution of CD1⁺ T lymphocytes to the staining patterns in both diseases.

NKT Cells Are Present in TCR Leprosy but Not in Sarcoidosis Patients

Human NKT cells are T cells with NK cell surface markers, using an invariant V α 24-J α 18 TCR α chain, associated preferably with the V β 11 chain and restricted by the MHC1b CD1d molecule. Their murine V α 14-J α 281 counterparts have been shown to be necessary for the development of the granulomatous lesions that follow the injection of deproteinized mycobacterial cell walls.²⁰

All samples were assayed for the presence of V α 24⁺ transcripts, followed by the research of the NKT-specific, V α 24-J α 18 transcripts.²⁸ The V α 24-C α PCR products were studied by primer extension, using a fluorescent C α -specific probe. Patients T1, T5, R1, and R2 displayed a Gaussian-like distribution of their CDR3 length; patients T2-T4 patients displayed a unique peak with a size compatible with that of the invariant chain of the TCR α chain of NKT cells (Figure 3a). On primer extension with the V α 24-J α 18 clonotypic probe, all TT and RR samples showed the presence of NKT cells. The resulting peak was superimposable on the V α 24-C α products in patients T2-T4, showing that NKT cells are predominant among the V α 24⁺ T cells of these patients.

In contrast, none of the sarcoidosis or lepromatous leprosy patients showed evidence of an infiltration by

Figure 1. Staining patterns of CD1a, CD1b, CD1c, and CD1d expression in patient R2 (A-D) and S1 (E-H). Cells were stained with the adequate primary antibody, followed by a streptavidin-biotin immunoperoxidase reaction. Positive cells are stained in brown; counterstaining was performed with hematoxylin. Whereas staining for CD1b and CD1c was strongly associated with the granulomatous structures, CD1a also stained positive in the epidermis (epidermal dendritic cell marker), and CD1d showed additional association with basal layers of the keratinocytes. To identify the nature of the CD1d⁺ cells within the granulomas, serial sections were stained with various cell markers. CD1d⁺ cells were found to colocalize with CD83, a marker for the dendritic cell lineage (data not shown).

Figure 3. a: Immunoscope analysis of the Vα24-Cα (**left**) and the Vα24-Jα18 (**right**) amplification product in patients S5, T1, and T3. Whereas patients S5 and T1 show polyclonal distributions of the Vα24-positive population (indicated by the Gaussian or comb-like profile with various peaks), patient T3 shows infiltration by a T-cell population with homogeneous Vα24-Cα distribution, as indicated by the single peak expansion. For patients T1 and T3 the clonotypic runoff experiment with the NKT-specific Jα18 primer shows a single peak signal indicative of the Vα24-Jα18 rearrangement. Whereas in patient T3 the NKT population is superimposable on the Vα24⁺ population, in patient T1 this population represents only a subfraction of the total Vα24⁺ T-cell population. Patient S5 shows no positive signal in the Vα24-Jα18 runoff, indicating the absence of the particular NKT population in this patient. Sequence analysis showed for all T-cell-reactive leprosy patients the CDR3 sequence of the originally described clonotype (Vα24-TGT GTG GTG AGC GAC AGA-Jα18), with the exception of patient T3, who showed in addition a second sequence in which the serine was encoded by another triplet (Vα24-TGT GTG GTG TCT GAC AGA-Jα18). **b:** Localization of Vα24⁺ cells in the biopsy of patient R2 with a form of reversal leprosy. The arrows mark positive cells (brownish), which are located within the granulomatous structures, suggesting a recruitment by the invaded mycobacterial structures. Presence of the canonical Vα24-Jα18 rearrangement was confirmed by Immunoscope and sequence analysis.

NKT cells. In sarcoidosis patients S1, S5, and S6, we could detect a weak Vα24-Cα amplification product, but we could not detect the clonotypic Vα24-Jα18 transcript. Patients S2, S3, S4, and L1-L3 did not show any Vα24-Cα

amplification signal and were also found to be negative in the Vα24-Jα18 runoff experiments.

We then stained tissue sections, using a Vα24-specific monoclonal antibody raised against a Vα24-Jα18⁺ DN T-cell clone.^{32,33} No positive cells were found in any of the sarcoidosis or LL specimens, whereas two T-cell-reactive leprosy patients also scored positive in immunohistochemistry. Figure 3b shows the staining pattern for Vα24⁺ in patient R2.

To confirm the presence of NKT cells seen in the immunoscope analysis, we then cloned and sequenced the Vα24-Cα transcripts in all seven T-cell-reactive leprosy patients. Whereas we could find in all patients the canonical sequence Vα24-TGT GTG GTG AGC GAC AGA-Jα18, patient T3 showed, in addition to this sequence, a Vα24-TGT GTG GTG TCT GAC AGA-Jα18 rearrangement in which the serine at the Vα-Jα junction was encoded by a triplet (TCT) other than the germline transcribed triplet AGC, which is usually associated with the NKT cell population.²⁸ This finding argues for a TCR-mediated selection process within the lesion of this patient.

Thus classical Vα24-Jα18 NKT cells are present in T-cell-reactive leprosy lesions but are absent from cutaneous sarcoidosis lesions.

TCR α-Chain Usage in Leprosy Lesions

To further define the T cells detected by immunohistochemistry, we examined their TCR α-chain usage with a panel of 29 different primers covering almost 95% of the described Vα families.³⁴ The analysis of the α chain was preferred to that of the β chain because it allows simultaneously the characterization of the T-cell repertoire together with the screening for possible invariant TCR α chains.

In addition to the Vα24 segment, which we have shown to be mostly associated with NKT cells, all seven T-cell-reactive leprosy patients showed the preferential usage of either Vα6 and/or Vα14 (Figure 4). On Immunoscope analysis of the Vα6/14 -Cα PCR products with a Cα-specific primer, a Gaussian-like or, in some instances, a comb-like distribution of the TCR α-chain CDR3 profile was observed. The absence of dominant peaks is indicative of a polyclonal origin of the infiltrating T cells.

Single transcripts of the Vα6- and Vα14-Cα PCR products in various patients were cloned and sequenced to examine recurrency in their CDR3 region sequence (including the presence of invariant α chains) or biases in the usage of Jα segments.

We did not find invariant sequences within the analyzed clones; however, the DNA sequences showed a bias of the associated Jα, because Jα segments were preferentially associated with Vα6 and others were more often found to be associated with Vα14. They were segments Jα 4, 5 with Vα6 and Jα 30, 31, 32, 33, 34, 48, and 49 with Vα14. The Jα 1, 2, 14, 18, 24, 25, 28, 35, 36, 38, 46, 50, 51, 59, 60, 61 segments were not detected among the 220 sequences we have determined within the different leprosy patients (Figure 5A).

TCR V α chain usage in patients with cutaneous sarcoidosis and leprosy

Figure 4. The graph gives the usage of TCR-V α chains within the granulomatous lesions of patients with cutaneous sarcoidosis (S), tuberculoid leprosy (T), reversal leprosy (R), and lepromatous leprosy (L), from RT-PCR and the Immunoscope technique. Sarcoidosis patients showed unbiased V α usage with at least one single peak-shaped V α (■), which is suggestive of clonal populations. T-cell-reactive leprosy patients (T, R), however, showed a bias toward V α 6, V α 14, and V α 24, with a Gaussian-like or comb-like distribution of the CDR3 size (▨), which is suggestive of poly- or oligoclonal expansions. The V α 24⁺ expansions in patients T2, T3, and T4 were found to consist exclusively of V α 24-J α 18⁺ cells, as shown by clonotype experiments.

Figure 5. Usage of J α segments within the obtained V α 6 and V α 14 populations of T-cell-reactive leprosy (A) and sarcoidosis (B) patients. PCR products were cloned, and random clones were used for sequence analysis. Gray cells represent J α segments that were found only once within one patient; dotted cells indicate J α segments that were used in two or three analyzed sequences; black cells represent J α segments that were found four times or more (including recurrent sequences). J α segments indicated by open cells were not found at all. Preferential usage of J α segments in leprosy patients was observed for both V α 6 (J α 4, 5) and V α 14 (J α 30–34, 48, 49), a finding that was less dominant in cutaneous sarcoidosis.

Table 2. Obtained CDR3 Sequences (Amino Acid Code) within the Analyzed V α 6 and V α 14 Population in T-cell-Reactive Leprosy Patients and the Cutaneous Sarcoidosis Patients Expressing the Respective TCR α Chain within Their Lesions

V α 6	CDR3	Frequency	Jalpha
Patient T1			
FCAM	<u>SEDTGRRALT</u>	FG	J α 5
FCAM	<u>SADTGRRALT</u>	FG	J α 5
FCAM	RDGPPLKAAGNKLT	FG	J α 10
FWVE	AGSYQFT	FG	J α 19
FCAM	REGHSTYQLI	WG	J α 33
FCAM	RGIPSWAGGTSYGKLT	FG	J α 52
Patient T2			
FCAM	<u>RGDTGANSKLT</u>	FG	J α 56
FCAM	<u>REDTGANSKLT</u>	FG	J α 56
FCAM	REGFSGGYNKLI	FG	J α 4
FCAM	<u>RPDTGRRALT</u>	FG	J α 5
FCAM	RDGPPFKAAGNKLT	FG	J α 17
FCAM	REGTYNFNKFY	FG	J α 21
FCAM	REGRDDKII	FG	J α 30
Patient T3			
FOEG	<u>GRRALT</u>	FG	J α 5
FCAM	<u>REGQGYSTLT</u>	FG	J α 11
FCAM	RLNTGFQKLV	FG	J α 8
FCAM	<u>RDSGGADGLT</u>	FG	J α 45
FCAM	<u>RERSGGYNKLI</u>	FG	J α 4
FCAM	<u>RGGNDYKLS</u>	FG	J α 20
FCAI	<u>FGSARQLT</u>	FG	J α 22
FWPI	<u>FGSARQLT</u>	FG	J α 22
FCAM	<u>REGRVTNAGKST</u>	FG	J α 27
FCAM	<u>REGYYRDDKII</u>	FG	J α 30
FCAM	<u>RGANTGNQFY</u>	FG	J α 49
Patient T5			
FCAM	<u>SLSGGSYIPT</u>	FG	J α 6
FCAM	<u>REPNTGNQFY</u>	FG	J α 49
FCAM	<u>REELPGNQFY</u>	FG	J α 49
FCAM	<u>RDPSGNTGNQFY</u>	FG	J α 49
FCAM	<u>REFNTGNQFY</u>	FG	J α 49
FCAM	<u>REGRNAGNMLT</u>	FG	J α 39
FCAM	<u>REGELTGGGNKLT</u>	FG	J α 10
FCAM	<u>REGYSSASKII</u>	FG	J α 3
FCAM	<u>REEAGGYNKLI</u>	FG	J α 4
FCAM	<u>REGTRGSYIPT</u>	FG	J α 6
FCAI	<u>SARGNNRLA</u>	FG	J α 7
FCAM	<u>REGNNAGNTLT</u>	FG	J α 11
FCAM	<u>REVTAGTALI</u>	FG	J α 15
FCAM	<u>REVRAGTALI</u>	FG	J α 15
FCAM	<u>SAPNQAGTALI</u>	FG	J α 15
FCAM	<u>REGRAAGNKLT</u>	FG	J α 17
FCAM	<u>IYNQGGKLI</u>	FG	J α 23
FCAL	<u>DPSYNQGGKLI</u>	FG	J α 23
FCAM	<u>RGSLNSGNTPLV</u>	FG	J α 29
FCAM	<u>RDDDKII</u>	FG	J α 30
FCAM	<u>REGPSNTGKLI</u>	FG	J α 37
FCAM	<u>REDSNSGYALN</u>	FG	J α 41
FCAM	<u>REGRYGGSQGNLI</u>	FG	J α 42
FCAI	<u>PRDGGSQGNLI</u>	FG	J α 42
FCAM	<u>SFYGNKLV</u>	FG	J α 47
FCAM	<u>RGSNTGNQFY</u>	FG	J α 49
FCAM	<u>REFPSYTGNQFY</u>	FG	J α 49
FCAM	<u>REGIGAGGTSYGKLT</u>	FG	J α 52
Patient S1			
FCAM	<u>REPFNQGGKLI</u>	FG	J α 23
FCAM	<u>REGAGGSTLGRL</u>	FG	J α 18
FCAM	<u>REYLGNNRLA</u>	FG	J α 7
FCAM	<u>REYTGGGNKLT</u>	FG	J α 10
FCAM	<u>REAFSDGHKLL</u>	FA	J α 16
FCAV	<u>REPFNQGGKLI</u>	FG	J α 23
FCAM	<u>RERYNQGGKLI</u>	FG	J α 23
FCAM	<u>RERDNYGQNFV</u>	FG	J α 26

The CDR3 regions shows variability with recurrent sequences in every patients suggesting rather oligoclonality than a totally unbiased repertoire. Recurrent or highly similar sequences which were found more than four times in a single patient are underlined. Similar sequences between different patients (T1 and T2) are given in bold letters. All sequences which were isolated several times were found identical at the nucleotide level. (*Table 2 continues*)

Table 2. Continued

Vα 6	CDR3	Frequency	Jαpha
FCAM	REAGYGGATNKLI	FG	Jα 32
FCAM	REPFNAGNMLT	FG	Jα 39
Patient S2			
FCAM	<u>RGLHTGGGNKLT</u>	FG	Jα 10
FCAM	<u>REGGGADGLT</u>	FG	Jα 45
FCAM	RGLRTGGGNKLT	FG	Jα 10
Patient S5			
FCAM	<u>RENAGGTSYGKLT</u>	FG	Jα 52
FCAM	<u>YGNKLV</u>	FG	Jα 47
FCAM	RRDTGRRALT	FG	Jα 5
FCAI	GKAAGNKLT	FG	Jα 17
FCAR	REALTRGSARQLT	FG	Jα 22
FCAM	KTGANNLF	FG	Jα 36
FCAM	REAHLLYNQGGKLI	FG	Jα 37
FCAM	REGGAGNMLT	FG	Jα 39
FCAM	RCLSMR	FG	Jα 43
FCAM	VGNQFY	FG	Jα 49
FCAM	RVNAGGTSYGKLT	FG	Jα 52
FCAM	REDNAGGTSYGKLT	FG	Jα 52
FCAM	REGTSYGKLT	FG	Jα 52
FCAM	IIQGAQKLV	FG	Jα 54
FCAI	IIQGAQKLV	FG	Jα 54
FCAM	RLLQGAQKLV	FG	Jα 54
FCAM	RESRIQGAQKLV	FG	Jα 54
FCAK	PEYTGANSKLT	FG	Jα 56
Patient T1			
FCAF	<u>NSNSGYALN</u>	FG	Jα 41
FCAR	GDTDCLI	FG	Jα 34
FCAF	MKPSGYSTLT	FG	Jα 12
FCAY	KWGNFNKFY	FG	Jα 21
FCAY	ETAGNKLT	FG	Jα 17
FCAY	RFQSSGNTPLV	FG	Jα 29
FCAF	STPYGDDTPLV	FG	Jα 29
FCAF	RNNNARLM	FG	Jα 31
FCAY	KGIMDSNYQLI	WG	Jα 33
FCAR	GDTDCLI	FG	Jα 34
FCAF	MDSDKLI	FG	Jα 34
FCAF	TANSNSGYALN	FG	Jα 41
FCVT	GTASKLT	FG	Jα 44
FCAY	RGPNTGNQFY	FG	Jα 49
FCAY	RSTKNSGGSNYKLT	FG	Jα 53
FCAY	RSGASGANSKLT	FG	Jα 56
FCAY	RSKVQTGANSKLT	FG	Jα 56
Patient T3			
FCAF	FTGGGNKLT	FG	Jα 10
FCAY	<u>RIGYGGATNKLI</u>	FG	Jα 32
FCAR	GSNYKLT	FG	Jα 53
FCAY	RRETSGSRVT	FG	Jα 13
FCAY	RSSFNDYKLS	FG	Jα 20
FCAF	MKHRDDKII	FG	Jα 30
FCAY	RDSNYQLI	WG	Jα 33
FCAR	GSNYKLT	FG	Jα 33
FCAF	MNHPTDKLI	FG	Jα 34
FCAY	RSVRYGGSQGNLI	FG	Jα 42
FCAY	RSAYYGGSQGNLI	FG	Jα 42
FCAP	PKISNFGNEKLT	FG	Jα 48
FCAY	RGSSNQFY	FG	Jα 49
FCAY	RSPGDGGSNYKLT	FG	Jα 53
FCAY	GDGGAQKLV	FG	Jα 55
FLCF	GDGGAQKLV	FG	Jα 55
FCDT	GANSKLT	FG	Jα 56
FRVP	SWEYGNKLV	FG	Jα 47
Patient T4			
FCAY	<u>GNNNDMR</u>	FG	Jα 43
FCAF	ILNNNAGNMLT	FG	Jα 39
FCAL	MKSSNYQLI	WG	Jα 33
FCAF	SHFGNEKLT	FG	Jα 48
FCAY	RRGNNDMR	FG	Jα 43

(Table 2 continues)

Table 2. Continued

Vα 6	CDR3	Frequency	Jαpha	
FCAF	MKHVTDDKII	FG	1×	Jα 30
FCAY	RSPNNNARLM	FG	1×	Jα 31
FAAH	TGGATNKLI	FG	1×	Jα 32
FCAY	RSPPFGNNAGNMLT	FG	1×	Jα 39
FCAY	RNYGGSQGNLI	FG	1×	Jα 42
FCAY	RRGYYGGSQGNLI	FG	1×	Jα 42
FCAP	DGLT	FG	1×	Jα 45
FCAY	RTGGIFNFGNEKLT	FG	1×	Jα 48
FCAF	MKPWNTGNQFY	FG	1×	Jα 49
Patient R2				
FCAY	RSLVYNQGGKLI	FG	3×	Jα 23
FCAY	RSHDNYGQNFV	FG	3×	Jα 26
FCAY	RGTFGSTSGSRLT	FG	3×	Jα 58
FCAY	RRLNRDDKII	FG	2×	Jα 30
FCAF	MKQAGNQFY	FG	2×	Jα 49
FCAF	PILTGGGNKLT	FG	1×	Jα 10
FCAS	YSGGYQKVT	FG	1×	Jα 13
FCAF	MQGSARQLT	FG	1×	Jα 22
FCAY	RSSGAGKST	FG	1×	Jα 27
FCAF	MKLLMNRDDKII	FG	1×	Jα 30
FCAY	KNRDDKII	FG	1×	Jα 30
FCAY	RSADNNARLM	FG	1×	Jα 31
FCAF	MNGDGATNKLI	FG	1×	Jα 32
FCAY	RRSTDCLI	FG	1×	Jα 34
FCAY	RSPPFGNNAGNMLT	FG	1×	Jα 39
FCAY	RSPVNSGTYKYI	FG	1×	Jα 40
FCAY	HRDNDMR	FG	1×	Jα 43
FCAF	AEYGNKLV	FG	1×	Jα 47
FRAY	SGYGNKLV	FG	1×	Jα 47
FCAY	RCRMEYGNKLV	FG	1×	Jα 47
FCAF	SNFGNEKLT	FG	1×	Jα 48
FCAY	RIPWDFGNEKLT	FG	1×	Jα 48
FCAY	RSVHNSGGSNYKLT	FG	1×	Jα 53
FCAY	RRGIQGAQKLV	FG	1×	Jα 54
FCAY	RSARIQGAQKLV	FG	1×	Jα 54
FCAF	MSRGAQKLV	FG	1×	Jα 54
FCAA	AAQKLV	FG	1×	Jα 54
FCAY	RTRYTGANSKLT	FG	1×	Jα 56
FCAY	RRLLSAGANSKLT	FG	1×	Jα 56
FAAH	TGANSKLT	FG	1×	Jα 56
FCAY	RIWGGSEKLV	FG	1×	Jα 57
FCAY	REETSGSRLT	FG	1×	Jα 58
Patient S1				
FCAL	<u>YSGGGADGLT</u>	FG	15×	Jα 45
FCAS	MIYNQGGKLI	FG	1×	Jα 23
FCAY	RKLSNYGQNFV	FG	1×	Jα 26
FCAY	LNGGATDKLI	FG	1×	Jα 34
FCAY	RRGTTSGTYKYI	FG	1×	Jα 40
FCAY	RSSNSGYALN	FG	1×	Jα 41
FCPI	QGAQKLV	FG	1×	Jα 54
Patient S5				
FWAG	<u>YTGTGSKLT</u>	FG	8×	Jα 44
FCAL	<u>KIGFGNVLH</u>	CG	8×	Jα 35
FCAF	MRGNQGGKLI	FG	1×	Jα 23
FCAY	QDRDDKII	FG	2×	Jα 30
FCAF	MNYGGSQGNLI	FG	2×	Jα 42
FCAY	RSAGSQGNLI	FG	1×	Jα 42
FCAF	MKQTGTASKLT	FG	2×	Jα 44
FCGY	YSGGSNYKLT	FG	1×	Jα 53
Patient S6				
FCAY	<u>RSAGVTGTASKLT</u>	FG	22×	Jα 44
FCAY	<u>RSATGTASKLT</u>	FG	4×	Jα 44
FCAL	GSARQLT	FG	1×	Jα 22
FMPI	TDSWGKLQ	FG	1×	Jα 24
FCAY	RNNNARLM	FG	2×	Jα 31
FCAY	RSANTDKLI	FG	1×	Jα 34
FCAY	RPGYALN	FG	2×	Jα 41
FMNY	GGSQGNLI	FG	1×	Jα 42
FCAY	RAGTGNQFY	FG	1×	Jα 49
FCAF	IPIQGAQKLV	FG	1×	Jα 54

When we compared the isolated sequences in detail, we found no identical sequences between different patients. The recurrent finding of the same or a highly similar sequence within the different clones of a patient (V α 6 in patients T1, T2, and T5) could indicate a dominant sequence within an otherwise polyclonal pattern. Moreover, one rearrangement sequence (V α 6-J α 5) was found to be quite similar in CDR 3 size and amino acid sequence in patients T1 and T2, which could be suggestive of a common antigenic structure that selected the similar clones in the two patients (Table 2).

DN and CD4⁺/CD8⁺ human T cells have consistently been shown to react with mycobacteria-derived lipophilic antigens with no significant restriction in their TCR $\alpha\beta$ -chain usage.³⁵ However, the V α 7.2-J α 33, the V α 4-J α 29, and the V α 19-J α 48 rearrangements have been described to be preferentially associated with DN or CD4⁺/CD8⁺ T cells.^{28,29,36} As immunohistochemical analysis of the DN population was not found to be a suitable screening technique, we decided to search for the described invariant TCR α rearrangements by PCR and Immunoscope techniques. Fluorescent primers coding for the J α 33, J α 29, and J α 48 segments were designed and used for runoff experiments of the V α 7-C α , V α 4-C α , and V α 19-C α PCR products. No signal was observed in the material recovered from the T-cell-reactive leprosy lesions, showing the absence of V α -invariant T cells other than the V α 24-invariant NKT cell population.

Thus the local $\alpha\beta$ T-cell response in cutaneous leprosy consists chiefly of NKT cells and oligoclonal/polyclonal V α 6/V α 14 T cells.

TCR α -Chain Usage in Cutaneous Sarcoidosis Lesions

All biopsies of cutaneous sarcoidosis granulomas were studied in a similar way. We have already shown the absence of NKT cells by RT-PCR. On agarose gel electrophoresis of the V α -C α PCR products, and in contrast with what we observed in leprosy, all patients with cutaneous sarcoidosis showed the usage of a limited number of individual V α chains (except patient S1, who yielded usage of 28 of the screened 29 TCR α chains) within their granulomatous lesions, without any markable bias toward the usage of a particular V α region. It is worth noting that, as previously reported for pulmonary specimens,³⁷ we found in five of six patients an amplification for V α 2 in cutaneous sarcoidosis lesions (Figure 4).

On immunoscope analysis of the distribution of the CDR3 length of the different TCR α chains in all patients, we found Gaussian-like distributions of the V α -C α peaks, representing polyclonal expansions of the infiltrating T lymphocytes, as well as distorted profiles with dominant peaks, for V α chains that were different in each patient and indicated the presence of T-cell populations with a conserved length of the CDR3 region of the TCR α chain, correlating with oligo- or monoclonal expansions. In this respect, the patterns observed in cutaneous sarcoidosis are very reminiscent of those observed in a number of

human diseases involving antigen-driven T-cell proliferation.²⁷

We determined the sequence of the most dominating expansions in each patient, either by direct sequencing (which proved successful in patients S1 and S4) or following the cloning of the V α -C α PCR product and determination of the expanded sequence (S2, S3, S5, and S6) by sequencing several clones. In four patients (S1, S2, S3, and S4) a single sequence of the CDR3 region typical for clonal expansions was recurrently determined. The sequences obtained from the different patients showed no similarities at the amino acid level, even when the same V α was studied (V α 9 for patients S1 and S2) (Table 3).

Some "peaks" were not of clonal origin: we could not identify a predominant CDR3 sequence within the 10-aa peak of V α 20 in patient S5 and within the 10-aa expansion of V α 16 in patient S6. The comparison of the CDR3 amino acid sequences, however, was reminiscent of the recognition of the same antigen in a given patient: in patient S5 the CDR3 sequences had a very similar sequence containing a CLVG-X-X-DTG motif (16 of 19) or a motif in which D-T was replaced by another hydrophilic amino acid (S or G); in addition, 10 of 19 of the 10-aa CDR3-containing sequences were found to include the J α 5 chain. Although the similarities were less striking in the CDR3 region analysis of the V α 16-C α amplification in patient S6, we still found some common features shared by most amino acid sequences (Table 3).

To compare for possible similarities between the T cells invading sarcoidosis and leprosy at a molecular level, we extended the sequence analysis of the CDR3 region in the V α 6⁺ and V α 14⁺ T-cell populations to the sarcoidosis patients showing infiltration of the respective chains, who were S1, S2, and S5 for V α 6 and S1, S5, and S6 for V α 14. This analysis showed, in parallel to the T-cell-reactive leprosy patients, an oligoclonal pattern with few clones on expansion in an otherwise polyclonal composition of the V α 6⁺ or V α 14⁺ T-cell populations (Table 2). When comparing for the association with various J α chains, we did not find the same segments preferably used in cutaneous sarcoidosis as in leprosy (eg, J α 33 was not used at all in sarcoidosis but was used in several leprosy specimens); still a preferred pairing of the two analyzed TCR α chains could be observed (Figure 5B).

Using the Immunoscope technique and the V α 7.2-J α 33-, the V α 4-J α 29-, and the V α 19-J α 48-specific clonotypic primers, we found that patients S1 and S5 showed a clonotypic peak of 190 bp (indicative of the V α 7.2-J α 33 rearrangement), and S1 showed the 196-bp peak indicative of the V α 19-J α 48 rearrangement. The canonical V α 4-J α 29 rearrangement was not detected in any of the patients. To rule out a bias on the level of J α usage, we looked for transcription of the J α segments 18 and 33 in all identified V α chains. These experiments showed that the use of the segments J α 18, J α 29, J α 33, and J α 48 was not restricted to the populations of V α 4⁺, V α 7⁺, V α 19⁺, or V α 24⁺ cells as identified by runoff experiments (data not shown) with all identified V α chains in the sarcoidosis patients and sequence analysis of the V α 6 and V α 14 populations (Table 2).

Table 3. Selected Single Peaks Suggestive of Clonal Expansions Sequenced in Sarcoidosis Patients

V alpha	CDR3	J alpha	Frequency
Patient S1 V α 9	FCAVSCVSGGYNKLI	FG J α 4	DS
Patient S2 V α 9	FCALSRQAASKLT	FG J α 44	9/9
Patient S3 V α 2	FCVAYLMNRDDKII	FG J α 30	12/12
Patient S4 V α 22	FCALLSGAGSYQLT	FG J α 28	DS
Patient S5 V α 20	FCLVGDRTNFGNEKLT	FG J α 8	1/26
	FCLVGATDTGRRALT	FG J α 5	4/26
	FCLVGHMDTGRRALT	FG J α 5	5/26
	FCLVGSMDTGRRALT	FG J α 5	1/26
	FCLVGAPDTGGFKTI	FG J α 9	6/26
	FCLVGPPASGGSYIPT	FG J α 6	2/26
	FCLVGDRSTGFQKLIV	FG J α 8	1/26
	FCLVEVTGTASKLT	FG J α 44	1/26
	FCLVGDAQGTALI	FG J α 15	1/26
	FCLVSGSARQLT	FG J α 22	1/26
Patient S6 V α 16	FCAVRDGTQAGTALI	FG J α 15	4/13
	FCAVREIKAAGNKLT	FG J α 17	4/13
	FCAVSPPPQGSNYQLI	WG J α 33	2/13
	FCAVRDMANAGKST	FG J α 27	3/13

Patients S1–S4 showed unique expansions, whereas patient S5 and S6 showed a composition of various CDR3 sequences that yielded a high degree of similarity in patient S5 (underlined amino acids) and, to a lesser degree, in patient S6, suggesting a selection of similar clones within the lesions of these patients.

Discussion

The formation of granulomatous skin reactions is a well-known feature shared by T-cell-reactive leprosy and cutaneous sarcoidosis. As a very similar composition of the cellular subsets is found in the two diseases (eg, macrophages, dendritic cells and lymphocytes, which surround and infiltrate an inflammatory granulomatous site), it has been proposed for a long time that granuloma formation in sarcoidosis is induced in a fashion similar to that of leprosy in response to an antigenic stimulation of mycobacterial origin (reviewed in ref 13). Conversely, if “atypical” mycobacteria are associated with the development of sarcoidosis granulomas, the T-cell responses in sarcoidosis and T-cell-reactive leprosy are expected to share traits in common.

The samples we have collected possess the immunohistochemical features already described⁴ and are thus characteristic of the different forms of leprosy as well as of cutaneous sarcoidosis, and the cell content of the sarcoidosis lesions mimics that of T-cell reactive leprosy. However, the T-cell responses in these two families of diseases, analyzed by a combination of RT-PCR, Immunoscope, and systematic DNA sequencing carried out on material with similar T-cell content, appear to be profoundly different. First, NKT cells are present among the T cells infiltrating T-cell-reactive leprosy lesions but are absent from sarcoidosis lesions. Second, the immune response in leprosy is dominated by a polyclonal V α 6 and V α 14 usage, whereas the immune response in cuta-

neous sarcoidosis is dominated by a mixture of polyclonal infiltrates associated with monoclonal or oligoclonal cell expansions, reminiscent of an antigen-driven proliferation of T cells and profoundly different from the patterns observed in the presence of mycobacteria. The oligoclonality we have observed in cutaneous sarcoidosis patients correlates well with previous analyses of pulmonary granulomas,^{22,38,39} although a restricted repertoire has not been found in all studies.⁴⁰ Oligoclonality cannot be due to the limited amount of material, inasmuch as we have observed a large panel of V α segments in patient S1, who otherwise did not show higher percentages of invading T cells; moreover, the Immunoscope technique has been shown to detect one cell in 10⁵ cells,⁴¹ a sensitivity sufficient to identify even very minor lymphocytic populations.

However, biases in the cutaneous TCR repertoire may be associated with the processes of homing to inflamed skin, which are highly complex and require a variety of cellular interactions favoring activated and/or antigen-specific T-cell populations.⁴² In this respect, Klein and co-workers showed a restricted V β usage with oligoclonal expansions within the sites of Kveim reagent injections, a diagnostic reaction appearing 4 weeks after intradermal injection of extracts of sarcoidosis spleen or lymph nodes, which leads to granuloma formation virtually identical to that of primary cutaneous sarcoidosis.⁴³ The expansions identified in our patients were seen within different V α -C α expansions and revealed CDR3 shapes with only limited similarities between the clones present in

different individuals, thus suggesting an individual predisposition to a yet unidentified antigenic stimulus. This idea is further supported by the observations we made in patients S5 and S6, where the peak-shaped sequences were not identical but contained a limited number of similar sequences, which could well have been stimulated by a single antigen. In conclusion, our results suggest that altogether different mechanisms underlie the development of granulomas in T-cell-reactive leprosy and reversal leprosy and in cutaneous sarcoidosis, and this may be taken as meaning that (atypical) mycobacteria do not contribute to the progression of cutaneous sarcoidosis. Moreover, were it to be proved that the observed expansions are locally induced by some antigen-driven process, the presence of oligoclonally expanded T cells in the lesions leads to the conclusion that a systematic search for sarcoidosis-associated antigens, whether of self or nonself origin, could be a fruitful alternative approach to the etiology of the disease.

The T-cell response in leprosy lesions is worth discussing. In the investigated patients with leprosy we found the well-known differences in APC and T-cell infiltration between the T-cell-reactive forms with a high immunocellular content and the lymphocyte-poor form of lepromatous leprosy. This expected finding represents one of the criteria for the classification of leprosy patients.²⁴ When analyzing the V α repertoire of the patients with TT and RR, we found a striking bias of the α chains used *versus* V α 6, V α 14, and V α 24. Because major differences in the efficiency of the primers used were ruled out,³⁴ only three V α segments are predominantly used by the T cells infiltrating T-cell-reactive leprosy patients. The strong bias *versus* V α 6 and V α 14 was somehow surprising, although Strohal had shown in earlier studies that T cells using these two V α segments are preferentially found in normal skin.⁴⁴ The V α 6 and V α 14 T cells of all lesions were polyclonally expanded in terms of CDR3 length, amino acid sequence, and J α usage. No invariant chain could be identified on the basis of identical recurrent sequences and unique J α usage. The polyclonal expansion of cells using solely V α 6 and 14 is difficult to account for. The bias in the V β usage (V β 6,12,14,19) previously described in leprosy patients⁴⁵ associated with the currently reported selective usage of V α 6 and V α 14 (two α segments that are structurally related)⁴⁶ may reveal a particular response to a nominal mycobacterial antigen. However, one would expect a highly skewed diversity within the expanded α chains that is due to an antigen-driven process or to preferential selection of an invariant α chain and thus a constant J α usage, none of which was observed in the present studies. The usage of V α 6 and V α 14 segments may also reveal the stimulation of T cells by some TCR α -specific superantigen secreted by proliferating mycobacteria as described for TCR β chains.⁴⁷ As TCR α -specific superantigens have not yet been identified, this remains questionable, but the contribution of the α chain to superantigen recognition is currently under debate, with some authors favoring an important role in TCR-superantigen interaction.^{48,49}

The analysis of the V α 24⁺ cells showed, in most of our T-cell-reactive leprosy patients, a disturbed distribution

of the Immunoscope profile with an expanded peak corresponding to the CDR3 length of the known V α 24-J α 18 clonotype. The presence of this clonotype, which is typical of human NKT cells, was further confirmed by J α 18-specific runoff experiments and sequence analysis, which showed an amplification product in all T-cell-reactive but not in lepromatous leprosy patients. The presence of NKT cells in leprosy granulomas had not previously been described, and the presence of this distinctive T-cell subset is clearly associated with T-cell-reactive forms of leprosy. The finding that the invading NKT cell population at least in one patient displayed differences at the nucleotide level in their V α 24-J α 18 transcript with an identical sequence at the amino acid level enforces the idea that NKT cells in the leprosy granulomas have undergone a selection process.

The V α 24-J α 18 TCR invariant NKT cells are restricted by the CD1d molecule and have a homolog in the murine V α 14-J α 281 TCR invariant NKT cells.⁵⁰ These cells have been found to carry markers of the NK lineage, such as CD161 or NK1.1 together with the $\alpha\beta$ TCR. They are predominantly found in the DN or CD4⁺CD8⁻ pool. The physiological role of the NKT cells in humans and mice is pleiotropic; they have been implicated in the induction of a Th2 immune response,⁵¹ the control of autoimmune disease,⁵² and an antitumoral activity after activation via interleukin-12 or specific ligands.⁵³⁻⁵⁵ In a murine model of granuloma development by mycobacterial cell wall components, these cells have recently been demonstrated to be almost exclusively recruited by glycolipids belonging to the phosphatidylinositolmannoside family and to be of crucial importance for granuloma formation.²⁰ However, an activation of NKT by CD1d⁺ cells supplemented with mycobacterial cell wall extracts has not yet been observed *in vitro*, and the nature of the activation process of NKT cells remains elusive. It is possible that the mycobacterial cell wall-induced NKT-cell-dependent granuloma formation is not dependent on mCD1; an alternative activation could be induced through lectin-like structures that are common to NK and NKT cells.⁵⁶ NKT cells were found in all patients with T-cell-reactive leprosy but not in lepromatous leprosy patients who lack a sufficient cellular immune response against *M. leprae*. They were not found in sarcoidosis patients, although CD1 molecules were detectable at high levels in the lesions. In any event, the formation of a granuloma in the absence of an obvious bacterial load, as in cutaneous sarcoidosis, clearly does not require the local recruitment of NKT cells. The identification of two further T-cell rearrangements previously described with DN T cells (V α 7.2-J α 33 and V α 19-J α 48) within the sarcoidosis lesions is difficult to explain. As no functional role for these TCR α -invariant cells has been described, their presence might be due to a yet unknown specific mechanism. However, as we found the V α 7.2-J α 33 rearrangement in only two of six and the V α 19-J α 48 rearrangement in only one of six patients, we would favor a nonspecific infiltration into the inflamed skin.

In contrast, the recruitment of NKT cells in T-cell-reactive leprosy strengthens the hypothesis that NKT cells have an important role in the innate immune response

toward substances produced by mycobacteria in the present case and by intracellular bacteria in general.^{57,58}

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References

- Constant P, Davodeau F, Peyrat M, Poquet Y, Puzo G, Bonneville M, Fournier J: Stimulation of human gd T cells by nonpeptidic mycobacterial ligands. *Science* 1994, 264:267–270
- Fournié J-J, Mullins RJ, Basten A: Isolation and structural characteristics of a monoclonal antibody defined cross reactive phospholipid antigen from *Mycobacterium tuberculosis* and *Mycobacterium leprae*. *J Biol Chem* 1991, 266:12111–12119
- Jullien D, Stenger S, Ernst W, Modlin R: CD1 presentation of microbial nonpeptide antigens to T cells. *J Clin Invest* 1997, 100:s29–s32
- Modlin R, Hofman F, Meyer P, Sharma O, Taylor C, Rea T: In situ demonstration of T lymphocytes subsets in granulomatous inflammation: leprosy, rhinoscleroma, and sarcoidosis. *Clin Exp Immunol* 1983, 51:430–438
- Sieling PA, Chatterjee D, Porcelli SA, Prigozy TI, Mazzaccaro RJ, Soriano T, Bloom BR, Brenner MB, Kronenberg M, Brennan PJ, Modlin RL: CD1-restricted T cell recognition of microbial lipoglycan antigens. *Science* 1995, 269:227–230
- Tanaka Y, Sano S, Nieves E, De Libero G, Rosa D, Modlin RL, Brenner MB, Bloom BR, Morita CT: Nonpeptide ligands for human gd T cells. *Proc Natl Acad Sci USA* 1994, 91:8175–8179
- Thole J, Janson A, Cornelisse Y, Schreuder G, Wiele B, Naafs B, de Vries R, Ottenhoff T: HLA-class II-associated control of antigen recognition by T cells in leprosy: a prominent role for the 30/31-kDa antigens. *J Immunol* 1999, 162:6912–6918
- Verhagen C, Faber W, Klatser P, Buffing A, Naafs B, Das P: Immunohistochemical analysis of in situ expression of mycobacterial antigens in skin lesions of leprosy patients across the histopathological spectrum—association of mycobacterial lipoarabinomannan (LAM) and *Mycobacterium leprae* phenolic glycolipid-I (PGL-I) with leprosy reactions. *Am J Pathol* 1999, 154:1793–1804
- Bergeron A, Bonay M, Kambouchner M, Lecossier D, Riquet M, Soler P, Hance A, Tazi A: Cytokine patterns in tuberculous and sarcoid granulomas—correlations with histopathologic features of the granulomatous response. *J Immunol* 1997, 159:3034–3043
- Chensue S, Warmington K, Ruth J, Sanghi P, Lincoln P, Kunkel S: Role of the monocyte chemoattractant protein-1 (MCP-1) in Th1 (mycobacterial) and Th2 (schistosomal) antigen-induced granuloma formation—relationship to local inflammation, Th cell expression, and IL-12 production. *J Immunol* 1996, 157:4602–4608
- Agostini C, Cassatella M, Zambello R, Trentin L, Gasperini S, Perin A, Piazza F, Siviero M, Faccio M, Dziejman M, Chilos M, Qin S, Luster A, Semenzato G: Involvement of the IP-10 chemokine in sarcoid granulomatous reactions. *J Immunol* 1998, 161:6413–6420
- El-Zaatari F, Naser S, Markesich D, Kalter D, Engstand L, Graham D: Identification of *Mycobacterium avium* complex in sarcoidosis. *J Clin Microbiol* 1996, 34:2240–2245
- Newman L, Rose C, Maier L: Sarcoidosis. *N Engl J Med* 1997, 336:1224–1234
- Popper H, Klemen H, Hoeffler G, Winter E: Presence of mycobacterial DNA in sarcoidosis. *Hum Pathol* 1997, 28:796–800
- Hance A: The role of mycobacteria in the pathogenesis of sarcoidosis. *Semin Res Infect* 1998, 13:197–205
- Vokurka M, Lecossier D, du Bois R, Wallaert B, Kambouchner M, Tazi A, Hance A: Absence of DNA from mycobacteria of the *M. tuberculosis* complex in sarcoidosis. *Am J Respir Crit Care Med* 1997, 156:1000–1003
- Beckman EM, Porcelli SA, Morita CT, Behar SM, Furlong ST, Brenner MB: Recognition of a lipid antigen by CD1 restricted ab+ T cells. *Nature* 1994, 372:691–694
- Beckman EM, Melian A, Behar SM, Sieling PA, Chatterjee D, Furlong ST, Matsumoto R, Rosat J, Modlin RL, Porcelli SA: CD1c restricts responses of mycobacteria-specific T cells. *J Immunol* 1996, 157:2795–2803
- Bendelac A, Rivera MN, Park SH, Roark JH: Mouse CD1-specific NK1 T cells: development, specificity, and function. *Annu Rev Immunol* 1997, 15:535–562
- Apostolou I, Takahama Y, Belmont C, Kawano T, Huerre M, Marchal G, Cui J, Taniguchi M, Nakauchi H, Fournie J-J, Kourilsky P, Gachelin G: Murine natural killer T-cells contribute to the granulomatous reaction caused by mycobacterial cell walls. *Proc Natl Acad Sci USA* 1999, 96:5141–5146
- Forman J, Klein J, Silver R, Liu M, Greenlee B, Moller D: Selective activation and accumulation of oligoclonal Vb-specific T cells in active pulmonary sarcoidosis. *J Clin Invest* 1994, 94:1533–1542
- Vissinga C, Springmeyer S, Concannon P: TCR expression and clonality analysis in pulmonary sarcoidosis. *Hum Immunol* 1996, 48:98–106
- Moller DR: T-cell receptor genes in sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 1998, 15:158–164
- Ridley DS, Jopling WH: Classification of leprosy according to immunity. A five-group system. *Int J Lepr Other Mycobact Dis* 1966, 34:255–273
- Pannetier C, Levraud JP, Lim A, Even J, Kourilsky P: The immunoscope approach for the analysis of T cell repertoires. The Antigen T Cell Receptor: Selected Protocols and Applications. Edited by JR Oksenberg. New York, Chapman and Hall, 1998, pp 287–325
- Pannetier C, Cochet M, Darche S, Casrouge S, Zaller M, Kourilsky P: The sizes of the CDR3 hypervariable regions of the murine T-cell receptor b chain vary as a function of the recombined germ-line segments. *Proc Natl Acad Sci USA* 1993, 90:4319–4323
- Even J, Lim A, Puisieux I, Ferradini L, Dietrich P-Y, Toubert A, Hercend T, Triebel F, Pannetier C, Kourilsky P: T-cell repertoires in healthy and diseased human tissues analysed by T-cell receptor b-chain CDR3 size determination: evidence for oligoclonal expansions in tumours and inflammatory diseases. *Res Immunol* 1995, 146:65–80
- Porcelli S, Yockey C, Brenner M, Balk S: Analysis of T cell antigen receptor (TCR) expression by human peripheral blood CD4–8–a/b T cells demonstrates preferential use of several Vb genes and an invariant TCR a chain. *J Exp Med* 1993, 178:1–16
- Han M, Harrison L, Kehn P, Stevenson K, Currier J, Robinson M: Invariant or highly conserved TCR a are expressed on double-negative (CD3+CD4–CD8–) and CD8+ T cells. *J Immunol* 1999, 163:301–311
- Sieling P, Jullien D, Dahlem M, Tedder T, Rea T, Modlin R, Porcelli S: CD1 expression by dendritic cells in human leprosy lesions: correlation with effective host immunity. *J Immunol* 1999, 162:1851–1858
- Nickoloff B, Wrone-Smith T, Bonish B, Porcelli S: Response of murine and normal human skin to injection of allogeneic blood-derived psoriatic immunocytes. *Arch Dermatol* 1999, 135:546–552
- Dellabona P, Casorati G, Friedli B, Angman L, Sallusto F, Tunnaccliff A, Roosneck E, Lanzavecchia A: In vivo persistence of expanded clones specific for bacterial antigens within the human T cell receptor a/b CD4–8–subset. *J Exp Med* 1993, 177:1763–1771
- Padovan E, Casorati G, Dellabona P, Meyer S, Brockhaus M, Lanzavecchia A: Expression of two T cell receptor a chains: dual receptor T cells. *Science* 1993, 262:422–424
- Genevee C, Diu A, Nierat J, Caignard A, Dietrich P, Ferradini L, Roman-Roman S, Triebel F, Hercend T: An experimentally validated panel of subfamily-specific oligonucleotide primers (V alpha 1-w29/V beta 1-w24) for the study of human T cell receptor variable V gene segment usage by polymerase chain reaction. *Eur J Immunol* 1992, 22:1261–1269
- Grant E, Degano M, Rosat J-P, Stenger S, Modlin R, Wilson I, Porcelli S, Brenner M: Molecular recognition of lipid antigens by T cell receptors. *J Exp Med* 1999, 189:195–205
- Tilloy F, Treiner E, Park S-H, Garcia C, Lemonnier F, Delasalle H, Bendelac A, Bonneville M, Lantz O: An invariant TCR-a chain defines a novel TAP-independent MHC class Ib-restricted ab T cell subpopulation in mammals. *J Exp Med* 1999, 189:1907–1921
- Grunewald J, Janson C, Eklund A, Oehrns M, Olerup O, Persson U, Wigzell H: Restricted Va2.3 gene usage by CD4+ T lymphocytes in bronchoalveolar lavage fluid from sarcoidosis patients correlates with HLA-DR3*. *Eur J Immunol* 1992, 22:129–135

38. Jones C, Lake R, Wijeyekoon J, Mitchell D, du Bois R, O'Hehir R: Oligoclonal V gene usage by T lymphocytes in bronchoalveolar lavage fluid from sarcoidosis patients. *Am J Respir Cell Mol Biol* 1996, 14:470–477
39. Forrester J, Wang Y, Ricalton N, Fitzgerald J, Loveless J, Newman L, King T, Kotzin B: TCR expression of activated T cell clones in the lungs of patients with pulmonary sarcoidosis. *J Immunol* 1994, 153: 4291–4302
40. Hoshino T, Suzuki R, Tsuruta Y, Matsutani T, Kikuchi M, Kawamoto M, Gouhara R, Shiraishi T, Mochizuki M, Itoh K, Oizumi K: Non-restricted T cell receptor (TCR)-Va and-Vb gene usage in patients with pulmonary sarcoidosis. *Clin Exp Immunol* 1997, 108:529–538
41. Lim A, Toubert A, Pannetier C, Dougados M, Charron DPK, Even J: Spread of clonal T-cell expansions in rheumatoid arthritis patients. *Hum Immunol* 1996, 48:77–83
42. Santamaria Babi L, Moser R, Perez Soler M, Picker L, Blaser K, Hauser C: Migration of skin-homing T cells across cytokine-activated human endothelial cell layers involves interaction of the cutaneous lymphocyte-associated antigen (CLA) the very late antigen-4 (VLA-4), and the lymphocyte function associated antigen-1 (LFA-1). *J Immunol* 1995, 154:1543–1550
43. Klein J, Horn T, Formann J, Silver R, Teirstein A, Moller D: Selection of oligoclonal Vb-specific T cells in the intradermal response to Kveim-Siltzbach reagent in individuals with sarcoidosis. *J Immunol* 1995, 154:1450–1460
44. Strohal R, Paucz L, Pehamberger H, Stingl G: T-cell repertoire of lymphocytes infiltrating cutaneous melanoma is predominated by Va specificities present in T-cells of normal human skin. *Cancer Res* 1994, 54:4734–4739
45. Wang X, Golkar L, Uyemura K, Ohmen J, Villahermosa L, Fajardo T, Cellona R, Walsh G, Modlin R: Selection of T lymphocytes bearing limited T-cell receptor b chains in the response to a human pathogen. *J Immunol* 1993, 151:7105–7116
46. Arden B, Clark SP, Kabelitz D, Mak TW: Human T-cell receptor variable gene segment families. *Immunogenetics* 1995, 42:455–500
47. Ohmen J, Barnes P, Grisso C, Bloom B, Modlin R: Evidence for a superantigen in human tuberculosis. *Immunity* 1994, 1:35–43
48. Andersen P, Lavoie P, Sekaly R-P, Churchill H, Kranz D, Schlievert P, Karjalainen K, Mariuzza R: Role of the T cell receptor a chain in stabilizing TCR-superantigen-MHC class II complexes. *Immunity* 1999, 10:473–483
49. Daly K, Nguyen P, Hankley D, Zhang W, Woodland D, Blackman M: Contribution of the TCR alpha-chain to the differential recognition of bacterial and retroviral superantigens. *J Immunol* 1995, 155:27–34
50. Lantz O, Bendelac A: An invariant T cell receptor a chain is used by a unique subset of major histocompatibility complex class I-specific CD4+ and CD4–CD8– T cells in mice and humans. *J Exp Med* 1994, 180:1097–1106
51. Yoshimoto T, Bendelac A, Watson C, Hu-Li J, Paul WE: Role of NK1.1+ T cells in a TH2 response and in immunoglobulin E production. *Science* 1995, 270:1845–1847
52. Wilson S, Kent S, Patton K, Orban T, Jackson R, Exley M, Porcelli S, Schatz D, Atkinson M, Balk S, Strominger J, Hafler D: Extreme Th1 bias of invariant Va24JaQ T cells in type 1 diabetes. *Nature* 1998, 391:691–694
53. Kawano T, Cui J, Koezuka Y, Toura I, Kaneko Y, Sato H, Kondo E, Harada M, Koseki H, Nakayama T, Tanaka Y, Taniguchi M: Natural killer-like nonspecific tumor cell lysis mediated by specific ligand-activated Va14 NKT cells. *Proc Natl Acad Sci USA* 1998, 95:5690–5693
54. Kawano T, Cui J, Koezuka Y, Toura I, Kaneko Y, Motoki K, Ueno H, Nakagawa R, Sato H, Kondo E, Koseki H, Taniguchi M: CD1d-restricted and TCR-mediated activation of Va14 NKT cells by glycosylceramides. *Science* 1997, 278:1626–1629
55. Cui J, Shin T, Kawano T, Sato H, Kondo E, Toura I, Kaneko Y, Koseki H, Kanno M, Taniguchi M: Requirement for Va14 NKT cells in IL-12 mediated rejection of tumors. *Science* 1997, 278:1623–1626
56. Exley M, Garcia J, Balk SP, Porcelli S: Requirements for CD1d recognition by human invariant Va24+, CD4–CD8– T cells. *J Exp Med* 1997, 186:109–120
57. Fairhurst RM, Wang C-X, Sieling PA, Modlin RL, Braun J: CD1 restricted T cells and resistance to polysaccharide-encapsulated bacteria. *Immunol Today* 1998, 19:257–259
58. Schofield L, McConville MJ, Hansen D, Campbell AS, Fraser-Reid B, Grusby MJ, Tachado SD: CD1d-restricted immunoglobulin G formation to GPI-anchored antigens mediated by NKT cells. *Science* 1999, 283:225–229